A similar case of an infant and her mother was identified in the Wales congenital hypothyroid screening program (2). The infant’s blood-spot TSH was 104 mIU/L at 7 days after birth, and on recall 7 days later, her serum free thyroxine (FT₄) was 22 pmol/L, total T₄ was 180 nmol/L, and TSH was 48 mIU/L. Because of these discrepant results of FT₄ and total T₄ within the reference intervals with an increased TSH that appeared to increase on dilution, thyroid function tests were done on the mother. Maternal serum FT₄ was 14 pmol/L, and this binding was inhibited by the addition of excess TSH. At age 7 months, the infant’s TSH concentrations were within the reference interval, whereas the mother’s TSH remained increased, and in 2 subsequent pregnancies, both infants had increased but factitious serum and blood-spot TSH concentrations. The cause of the increased TSH concentrations was concluded to be an IgG to TSH in the mother’s serum, which was acquired transplacentally by the infants. Serum immunoglobulins that bind TSH have been described (3–10), but they are rare, and only 1 case report of an antibody binding to follicle-stimulating hormone has been documented (11). In contrast, IgG binding to prolactin (macroprolactin) is common and well described, accounting for up to 26% of all cases of hyperprolactinemia (12). The prevalence of macroprolactinemia depends on the assay system, with some having only low reactivity, whereas others have a much higher reactivity with prolactin. TSH complexes with IgG may be very rare or may go unidentified because of low reactivity in TSH assays. It is intriguing to speculate that the prevalence of these complexes is higher than is supposed, accounting for some cases of a typical FT₄ with an increased TSH, so-called subclinical hypothyroidism. In a community study from the north of England, long-term follow-up over a period of 20 years of a cohort of randomly selected individuals revealed that the annual risk of developing hypothyroidism in women is 4.3% both when antithyroid antibodies are present and when TSH is increased (13). However, not all individuals with increased TSH develop hypothyroidism, and this group may include individuals who have TSH-IgG complexes in their sera. Thus, screening for TSH-IgG complexes in subclinical hypothyroid patients might be valuable.

In screening for congenital hypothyroidism by detecting factitious increases in TSH attributable to TSH-IgG complexes, heterophilic antibodies, or other immunoglobulin effects (14), it is imperative that in the follow-up of an increased blood-spot TSH, a serum sample from the mother be collected and analyzed at the same time as a sample from the infant (15) to detect any real, but transient, increase in TSH in the infant attributable to transplacentally acquired thyrotropin receptor-blocking antibody.

References

Rhys John
Department of Medical Biochemistry
University Hospital of Wales
Health Park
Cardiff CF14 4XW, United Kingdom
Fax 44-29-2074-8383
E-mail rhysjohn@cardiffandvale.wales.nhs.uk
DOI: 10.1373/clinchem.2006.070185

False-Negative Pregnancy Test in Hydatidiform Mole
To the Editor:
A 16-year-old girl (gravida 0, para 0) presented to the emergency department with a 2-week history of nausea, vomiting, vaginal spotting, and lower leg edema. On examination we found a palpable lower abdominal mass. The patient acknowledged recent sexual activity but denied having any sexually transmitted diseases.
Molar pregnancy was suspected because of the typical “snowstorm” appearance observed with ultrasound examination. The quantitative serum β-subunit of human chorionic gonadotropin (β-hCG) concentration was 746.20 IU/L (reference interval, <0.5 to 2.90 IU/L; reportable interval, 0.5–1000 IU/L). The result of a qualitative urine hCG test, however, was negative.

Human error was initially suspected as the cause of the negative result in the first qualitative urine hCG test. Two experienced and well-trained senior medical technologists repeated the urine hCG test twice, but the results were still negative. The patient’s nurse also indicated that she did not think there had been mislabeling of the urine sample. Because of this discrepancy, we had a discussion with the clinicians and concluded that a hook effect might be responsible for the negative urinary hCG result and for the relatively low serum β-hCG concentration. We therefore repeated the tests at a 1 to 10 000 dilution and obtained values for serum β-hCG and urinary hCG of 3 835 000 IU/L and 4 873 400 IU/L, respectively.

According to the manufacturer’s information of the Beckman Access 2 analyzer, a hook effect will occur when the serum β-hCG concentration exceeds 1 000 000 IU/L. The manufacturer’s information for the ACON urine hCG One Step Pregnancy Device (Format: FHC-102) does not provide any information about a hook effect, but hook effects occurring in a qualitative urine and a quantitative serum β-hCG assay have both been reported recently (1, 2).

In this case, we report a novel observation of hCG concentrations high enough to cause a hook effect in both qualitative and quantitative hCG assays. We diluted the urine sample to various concentrations (1:2, 1:4, 1:6, 1:8, and 1:10 dilutions) and performed qualitative analysis of urinary hCG on each (Table 1). The results showed no hook effect in the qualitative urine hCG test at ~487 000 IU/L, but a hook effect was evident at concentrations of 609 000 to 2 000 000 IU/L. There was complete signal elimination at a concentration of 4 000 000 IU/L.

According to the Beckman package insert, the hook effect occurs at concentrations >1 000 000 IU/L. We found no hook effect in the quantitative serum β-hCG at a concentration of 958 750 IU/L, but did observe a hook effect at concentrations of >1 917 500 IU/L to 3 835 000 IU/L (Table 1). On the basis of our findings, we suggest that, when hydatidiform mole is suspected, the urine sample should be diluted at least 1:10, particularly when the ACON urine hCG One Step Pregnancy Device (Format: FHC-102) is used, to avoid inaccurate urine hCG results.

Hydatidiform mole results from the overproduction by tissue that would typically develop into the placenta. The incidence of molar pregnancy demonstrates marked geographic and ethnic differences, ranging from a highest incidence of 1 in 120–400 pregnancies in Asian countries such as Taiwan, the Philippines, and Japan, to a lowest incidence of 1 in 1000–2000 pregnancies in Europe and the United States (3). This disorder is more common in young women and women nearing the end of their reproductive years (4). In addition, there is a 1% risk of having molar disease in a subsequent pregnancy after a molar pregnancy (5). The classic clinical presentation includes vaginal bleeding with abnormal growth in the size of the uterus. The diagnosis is typically established by markedly increased concentrations of β-hCG and the characteristic snowstorm appearance on ultrasound.

In conclusion, although modern assay methods have eliminated many laboratory errors, a high-dose hook effect might occur in urine hCG tests for hydatidiform mole. To avoid false-negative hCG results, appropriate sample dilution must be performed. Furthermore, the details of the hook effect should be clearly explained in the manufacturer’s instructions. Medical technologists and physicians must also be aware of the hook effect to accurately interpret urinary hCG and serum β-hCG test results. Our report identifies the concentrations at which the hook effect occurred for both of these hCG methods for the lot numbers tested. This knowledge may be useful to clinicians and clinical laboratory specialists to avoid delays in diagnosis and therapy.

References
5. Berkowitz RS, Im SS, Bernstein MR, Goldstein DP. Gestational trophoblastic disease: subse-

| Table 1. Detection values of qualitative urine hCG and estimated values of quantitative urine hCG and quantitative serum β-hCG for serial dilutions assayed on a Beckman Access 2 analyzer. |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Dilutions      | Serum           | Estimated quantitative concentration* | Observed qualitative result |
|                |                 | Observed quantitative result           | Urine            |
| None           | 3 835 000       | 746.2                                      | 4 874 400        |
| 1:2            | 1 917 500       | 566.2                                      | 2 436 700        |
| 1:4            | 958 750         | >1000                                      | 1 218 350        |
| 1:6            | 639 167         | >1000                                      | 812 233          |
| 1:8            | 479 375         | >1000                                      | 609 175          |
| 1:10           | 383 500         | >1000                                      | 487 340          |

* Based on measurement of 1:10 000 dilutions of serum and urine.
Neural Tube Defects Cannot Be Diagnosed Prenatally by Electrophoresis of Amniotic Fluid Transferrin Isoforms

To the Editor:

Prenatal diagnosis of neural tube defects (NTDs) is based on ultrasonography; but uncertainty exists in a few cases, and biochemical analysis of amniotic fluid (AF) is required. Electrophoresis of AF acetylcholinesterase is a specific and sensitive method for diagnosis of myelomeningocele (1,2). Although false-positive results are rare, another biochemical technique would be of great value.

Adult and infant cerebrospinal fluid (CSF) is characterized by a specific marker, asialotransferrin or $\beta_2$-transferrin, because it migrates more slowly in electrophoresis than $\beta_1$-tetrasialotransferrin, the main isoform in all biological fluids (3). The specific behavior of $\beta_2$-transferrin is used for the detection of CSF leakage from the subarachnoid space into the nasal or aural cavity (4,5). Assuming that CSF would leak from a myelomeningocele-affected fetus into the AF, we screened AF for asialotransferrin.

We undertook a retrospective study of 12 AF samples from NTD-affected fetuses (16–35 weeks of gestation) and 36 AF gestational age-matched controls. Serum controls were from newborns or fetuses (collected by in utero puncture at 22 and 32 weeks for fetal karyotyping), and CSF controls were from newborns or infants (normal biochemical and bacteriologic findings). Electrophoresis was performed on agarose with the Hydragel 6 CSF® assay (Sebia), followed by immunostaining with a polyclonal anti-transferrin antiserum conjugated to peroxidase (Sebia). Dilutions were used to obtain a transferrin concentration of $\sim$10 mg/L.

Control sera and AF (regardless of gestational age) produced the classic $\beta_1$-transferrin band corresponding to the tetrasialotransferrin isoform, whereas control CSF produced 2 bands, a major band of $\beta_1$ mobility and a lighter band of $\beta_2$ mobility, corresponding to the asialo isoform (Fig. 1). AF from fetuses with NTDs gave the same pattern as controls without detectable $\beta_2$-transferrin.

To find an explanation for this unexpected result, we compared fetal and newborn CSF electrophoretic patterns. Fetal CSF ($n = 8$; 20 to 35 weeks of gestation) was collected by cranial puncture after medical termination of pregnancy (terminal renal failure, skeletal abnormalities, or trisomy 21). We observed that the fetal CSF pattern depended on gestational age. Before 27 weeks, there was a single $\beta_1$ band, whereas from 32 weeks, a smear appeared between $\beta_1$ and $\beta_2$. However, CSF from a newborn delivered at 37 weeks of gestation displayed the classic CSF pattern, with 2 distinct transferrin bands, rather than this peculiar pattern.

This study indicates that electrophoresis of AF transferrin isoforms is inadequate for prenatal diagnosis of NTD. There are 2 possible explanations for the absence of the $\beta_2$ band: (a) inadequate sensitivity of the technique because of unknown in vivo dilution of CSF leaks in AF; and (b) an absence of asialotransferrin in fetal CSF. The CSF fetal patterns agree with this hypothesis, showing the progressive appearance of the $\beta_2$ brain isoform from the 32nd week of gestation. The processing of brain glycosylation appears to be mature at birth as the same pattern can be observed in newborn and adult CSF. Similar findings have been reported for the abnormal glycosylation of transferrin in carbohydrate-deficient glycoprotein syndrome (6). Blood samples from affected fetuses display the classic $\beta_1$ pattern of unaffected fetuses, suggesting a false negative. The carbohydrate-deficient pattern appears only after birth.

Although negative, the present findings may be of interest to pediatric physicians, particularly when they are using protein glycosylation abnormalities for prenatal diagnosis.

1. Department of Laboratory Medicine
Kaohsiung Medical University Hospital
Kaohsiung, Taiwan

2. Department of Obstetrics and Gynecology
Faculty of Medicine
College of Medicine
Kaohsiung Medical University
Kaohsiung, Taiwan

3. Department of Clinical Biochemistry
Faculty of Biomedical Laboratory Science
College of Health Sciences
Kaohsiung Medical University
Kaohsiung, Taiwan

† These authors contributed equally to this work.
* Address correspondence to this author at: Department of Clinical Biochemistry, Faculty of Biomedical Laboratory Science, College of Health Sciences, Kaohsiung Medical University Hospital, 100 Shih-Chuan 1st Rd., Kaohsiung, Taiwan. Fax 886-7-238-2669; e-mail Dr Jyh Jong at kmu.edu.tw.

DOI: 10.1373/clinchem.2006.068056

Fig. 1. Electrophoretic patterns of human transferrin isoforms after agarose electrophoresis and enzyme immunostaining.

Lanes: a, serum control; b, AF control; c, CSF control; d, AF from an NTD-affected fetus; e and f, CSF from fetuses at 27 and 35 weeks of gestation; g, CSF from a newborn delivered at 37 weeks of gestation. A single band of $\beta_1$ mobility is observed in serum, AF, and fetal CSF before 27 weeks of gestation. From 32 to 35 weeks of gestation, a smear appears between the $\beta_1$ and $\beta_2$ bands (lane f). CSF from newborns and infants exhibited the classic pattern with the $\beta_2$-asialotransferrin band.

Neural Tube Defects Cannot Be Diagnosed Prenatally by Electrophoresis of Amniotic Fluid Transferrin Isoforms

To the Editor:

Prenatal diagnosis of neural tube defects (NTDs) is based on ultrasonography; but uncertainty exists in a few cases, and biochemical analysis of amniotic fluid (AF) is required. Electrophoresis of AF acetylcholinesterase is a specific and sensitive method for diagnosis of myelomeningocele (1,2). Although false-positive results are rare, another biochemical technique would be of great value.

Adult and infant cerebrospinal fluid (CSF) is characterized by a specific marker, asialotransferrin or $\beta_2$-transferrin, because it migrates more slowly in electrophoresis than $\beta_1$-tetrasialotransferrin, the main isoform in all biological fluids (3). The specific behavior of $\beta_2$-transferrin is used for the detection of CSF leakage from the subarachnoid space into the nasal or aural cavity (4,5). Assuming that CSF would leak from a myelomeningocele-affected fetus into the AF, we screened AF for asialotransferrin.

We undertook a retrospective study of 12 AF samples from NTD-affected fetuses (16–35 weeks of gestation) and 36 AF gestational age-matched controls. Serum controls were from newborns or fetuses (collected by in utero puncture at 22 and 32 weeks for fetal karyotyping), and CSF controls were from newborns or infants (normal biochemical and bacteriologic findings). Electrophoresis was performed on agarose with the Hydragel 6 CSF® assay (Sebia), followed by immunostaining with a polyclonal anti-transferrin antiserum conjugated to peroxidase (Sebia). Dilutions were used to obtain a transferrin concentration of $\sim$10 mg/L.

Control sera and AF (regardless of gestational age) produced the classic $\beta_1$-transferrin band corresponding to the tetrasialotransferrin isoform, whereas control CSF produced 2 bands, a major band of $\beta_1$ mobility and a lighter band of $\beta_2$ mobility, corresponding to the asialo isoform (Fig. 1). AF from fetuses with NTDs gave the same pattern as controls without detectable $\beta_2$-transferrin.

To find an explanation for this unexpected result, we compared fetal and newborn CSF electrophoretic patterns. Fetal CSF ($n = 8$; 20 to 35 weeks of gestation) was collected by cranial puncture after medical termination of pregnancy (terminal renal failure, skeletal abnormalities, or trisomy 21). We observed that the fetal CSF pattern depended on gestational age. Before 27 weeks, there was a single $\beta_1$ band, whereas from 32 weeks, a smear appeared between $\beta_1$ and $\beta_2$. However, CSF from a newborn delivered at 37 weeks of gestation displayed the classic CSF pattern, with 2 distinct transferrin bands, rather than this peculiar pattern.

This study indicates that electrophoresis of AF transferrin isoforms is inadequate for prenatal diagnosis of NTD. There are 2 possible explanations for the absence of the $\beta_2$ band: (a) inadequate sensitivity of the technique because of unknown in vivo dilution of CSF leaks in AF; and (b) an absence of asialotransferrin in fetal CSF. The CSF fetal patterns agree with this hypothesis, showing the progressive appearance of the $\beta_2$ brain isoform from the 32nd week of gestation. The processing of brain glycosylation appears to be mature at birth as the same pattern can be observed in newborn and adult CSF. Similar findings have been reported for the abnormal glycosylation of transferrin in carbohydrate-deficient glycoprotein syndrome (6). Blood samples from affected fetuses display the classic $\beta_1$ pattern of unaffected fetuses, suggesting a false negative. The carbohydrate-deficient pattern appears only after birth.

Although negative, the present findings may be of interest to pediatric physicians, particularly when they are using protein glycosylation abnormalities for prenatal diagnosis.